Amendments to the Specification

- Please replace the passage found from page 41, line 26 to page 42 line 10 with the following passage (**please note that the addition only relates to the sequence identifier**);

SEQ ID NO.:1:

5' GTG GCG ACC CTT CCC AAA TCG GAT CTG GTT CCG CGT GGA TCC TCT AGA GTC GAC CTG CAG GCA TGC AAT GCT TAT TCC ATT AAT CAA AAG GCT TAT TCA AAT ACT TAC CAG GAG TTT ACT AAT ATT GAT CAA GCA AAA GCT TGG GGT AAT GCT CAG TAT AAA AAG TAT GGA CTA AGC AAA TCA GAA AAA GAA GCT ATA GTA TCA TAT ACT AAA AGC GCT AGT GAA ATA AAT GGA AAG CTA AGA CAA AAT AAG GGA GTT ATC AAT GGA TTT CCT TCA AAT TTA ATA AAA CAA GTT GAA CTT TTA GAT AAA TCT TTT AAT AAA ATG AAG ACC CCT GAA AAT ATT ATG TTA TTT AGA GGC GAC GAC CCT GCT TAT TTA GGA ACA GAA TTT CAA AAC ACT CTT CTT AAT TCA AAT GGT ACA ATT AAT AAA ACG GCT TTT GAA AAG GCT AAA GCT AAG TTT TTA AAT AAA GAT AGA CTT GAA TAT GGA TAT ATT AGT ACT TCA TTA ATG AAT GTT TCT CAA TTT GCA GGA AGA CCA ATT ATT ACA AAA TTT AAA GTA GCA AAA GGC TCA AAG GCA GGA TAT ATT GAC CCT ATT AGT GCT TTT CAG GGA CAA CTT GAA ATG TTG CTT CCT AGA CAT AGT ACT TAT CAT ATA GAC GAT ATG AGA TTG TCT TCT GAT GGT AAA CAA ATA ATA ATT ACA GCA ACA ATG ATG GGC ACA GCT ATC AAT CCT AAA TAA 3'

- Please replace the passage found at page 42, line 17 to line 30 with the following passage (**please note that the addition only relates to the sequence identifier**);

SEQ ID NO.:2:

1 GGATCCTCTA GAGTCGACCT GCAGGCATGC AATGCTTATT CCATTAATCA
51 AAAGGCTTAT TCAAATACTT ACCAGGAGTT TACTAATATT GATCAAGCAA
101 AAGCTTGGGG TAATGCTCAG TATAAAAAGT ATGGACTAAG CAAATCAGAA
151 AAAGAAGCTA TAGTATCATA TACTAAAAGC GCTAGTGAAA TAAATGGAAA
201 GCTAAGACAA AATAAGGGAG TTATCAATGG ATTTCCTTCA AATTTAATAA
251 AACAAGTTGA ACTTTTAGAT AAATCTTTTA ATAAAATGAA GACCCCTGAA
301 AATATTATGT TATTTAGAGG CGACGACCCT GCTTATTTAG GAACAGAATT

351 TCAAAACACT CTTCTTAATT CAAATGGTAC AATTAATAAA ACGGCTTTTG
401 AAAAGGCTAA AGCTAAGTTT TTAAATAAAG ATAGACTTGA ATATGGATAT
451 ATTAGTACTT CATTAATGAA TGTTTCTCAA TTTGCAGGAA GACCAATTAT
501 TACAAAATTT AAAGTAGCAA AAGGCTCAAA GGCAGGATAT ATTGACCCTA
551 TTAGTGCTTT TCAGGGACAA CTTGAAATGT TGCTTCCTAG ACATAGTACT
601 TATCATATAG ACGATATGAG ATTGTCTTCT GATGGTAAAC AAATAATAAT
651 TACAGCAACA ATGATGGGCA CAGCTATCAA TCCTAAATAA

- Please replace the second paragraph found at page 43, lines 6 to 10 with the following paragraph;

SEQ ID NO.: 3:

GSSRVDLQAC NAYSINQKAY SNTYQEFTNI DQAKAWGNAQ YKKYGLSKSE KEAIVSYTKS ASEINGKLRQ NKGVINGFPS NLIKQVELLD KSFNKMKTPE ~~NIMLFXGDDP~~ NIMLFRGDDP AYLGTEFQNT LLNSNGTINK TAFEKAKAKF ~~LNXDRLEYGY~~ LNKDRLEYGY ISTSLMNVSQ FAGRPIITKF KVAKGSKAGY IDPISAF**Q**GQ LEMLLPRHST YHIDDMRLSS DGKQIIITAT MMGTAINPK

- Please replace the passage found at page 43 starting at line 22 and ending at line 31 with the following passage ;

Ala (A) 18	7.9 %	
~~Arg (R) 6~~	~~2.6 %~~	Arg (R) 7 3.1 %
Asn (N) 18	7.9 %	
Asp (D) 10	4.4 %	
Cys (C) 1	0.4 %	
Gln (Q) 12	5.2 %	
Glu (E) 10	4.4 %	
Gly (G) 16	7.0 %	
His (H) 2	0.9 %	
Ile (I) 18	7.9 %	

- Please replace the passage found at page 44 starting at line 1 and ending at line 14 with the following passage ;

Leu (L) 17	7.4 %	
~~Lys (K) 23~~	~~10.0 %~~	Lys (K) 24 10.5 %
Met (M) 7	3.1 %	
Phe (F) 10	4.4 %	
Pro (P) 7	3.1 %	
Ser (S) 20	8.7 %	
Thr (T) 14	6.1 %	
Trp (W) 1	0.4 %	
Tyr (Y) 11	4.8 %	
Val (V) 6	2.6 %	
Asx (B) 0	0.0 %	
Glx (Z) 0	0.0 %	
~~Xaa (X) 2~~	~~0.9 %~~	Xaa (X) 0 0.0 %

- Please replace the paragraph found at page 45 from lines 16 to 32 with the following paragraph:

The kit portion of the illustrated system comprises a container ~~mean~~ means 1 for fibrinogen material, a container means 2 for thrombin material and a ~~container means~~4 container means 3 for a therapeutically active agent for facilitating axon growth (e.g. C3 or a modified or hybrid C3). If desired or necessary ~~the~~ the kit portion may include additional containers for the separate containment of other desired or necessary components; as shown the system in ~~figure~~ figure 9 includes in dotted outline an additional ~~container means~~ container means 4 for the flowable matrix forming part of the kit. The system also includes a mixing container 6 wherein the C3 (hybrid) is mixed with the matrix forming elements to form the supplemented flowable matrix forming carrier. The feed line 8 is indicative of the addition of C3 to the ~~container 8~~ container 6 whereas the feed ~~ligne~~ line 10 is indicative of the addition of the flowable

matrix forming elements from containers 1 and 2 and which is formed from the merging of feed lines 12 and 13. The mixing in the container means 6 may be effected or carried out in any suitable (known) fashion, (e.g. simple stirring with a magnetic ~~stirrer~~ stirrer). The output line 15 of the mixing container is indicative of the delivery of the supplemented mixture to the lesion site (e.g. by needle (e.g. syringe), pipette, [etc.] etc.).

Although in figure 9 the therapeutically active agent for facilitating axon growth (e.g. C3) is shown [As] as being associated with a separate ~~container 4~~, container means 3, if so desired or as necessary the

- Please replace the paragraph found at page 1 from lines 7 to 17 with the following paragraph:

The present invention provides methods for making, delivering and using formulations that combine a therapeutically active agent(s) (such as for example a Rho antagonist(s)) and a flowable carrier component capable of forming a therapeutically acceptable matrix in vivo (such as for ~~exa ple~~ example tissue adhesives), to injured nerves to promote repair and regeneration and regrowth of injured mammalian neuronal cells, e.g. for facilitating axon growth at a desired lesion site. ~~active~~ Active agents are known Rho antagonists such as for example C3, chimeric C3 proteins, etc. (see ~~blow~~ below) or substances selected from among known trans-4-amino(alkyl)-1-pyridylcarbamoylcyclohexane compounds (also see below) or Rho kinase inhibitors. The system for ~~exmple~~ example may deliver an antagonist(s) in a tissue adhesive such as[[.]] for example, a fibrin glue or a collagen gel to create a delivery matrix in situ. A kit and methods of stimulating neuronal regeneration are also included.

- Please replace the paragraph found at page 2 from lines 1 to 2 with the following paragraph:

In the following by way of example only reference will generally be made to axon growth at a [[a]] central nervous system (CNS) lesion site.

- Please replace the paragraphs found at page 2 from lines 6 to 22 with the following paragraphs:

It has been proposed to use various agents to stimulate regeneration of cut axons, i.e. nerve lesions. Please see for example canadian Patent application nos. 2,304,981 (McKerracher et al) and 2,300,878 (Stittmatter). These ~~document~~ documents propose the use of known Rho antagonists such as for example C3, chimeric C3 proteins, etc. (see ~~blow~~ below) as well as substances selected from among known trans-4-amino (alkyl)-1-pyridylcarbamoylcyclohexane compounds (also see below) or Rho kinase inhibitors for use in the regeneration of axons.

- Please replace the paragraphs found at page 7 from lines 1 to 25 with the following paragraphs:

patent no. 4,997,834 the entire contents of which are incorporated herein by references; this patent refers for example to compounds which may be selected from the group consisting of trans-4-aminomethyl-1-(4-~~pyridylcarbomoyl~~ pyridylcarbamoyl) cyclohexane,
trans-4-aminomethyl-trans-1-methyl-1-(4- pyridylcarbamoyl) cyclohexane,
trans-4-aminomethyl-cis-2-methyl-1-(4-pyridylcarbamoyl) cyclohexane,
trans-4-aminomethyl -1-(2-pyridylcarbamoyl) cyclohexane, trans-4-aminomethyl-1-(3-pyridylcarbamoyl) cyclohexane,
trans-4-aminomethyl-1[(3-hydroxy-2-pyridylcarbamoyl)] cyclohexane,
trans-4-aminomethyl-1-(3-methyl-4pyridylcarbamoyl) cyclohexane, 4-(trans-4-aminomethylcyclohexylcarboxamido)-2, 6-dimethyl-pyridine-N-oxide,
trans-4-aminomethyl-1-(2-methyl-4-pyridylcarbamoyl)cyclohexane,
trans-4-(2-aminoethyl)-1-(4-pyridylcarbamoyl) cyclohexane,
trans-4-(1-amino-1-methylethyl)1-(4-pyridylcarbamoyl) cyclohexane,
trans-4-(1-aminopropyl)-1-(4-pyridylcarbamoyl)cyclohexane, and pharmaceutically acceptable acid addition salts thereof.

Please also see also Ishizali et al. 2000. Molecular Pharmacology 57:976-983 3 which refers to Y-27632 in the dihydrochloride form as well as to a related compound Y-30141, namely (R)- [[trans--]] trans- 4-(1aminoethyl)-N-(1H-pyrrolo[2,3] pyridin-4-yl) cyclohexanecarboamide dihydrochloride. A patent application ~~A Medicines comparising~~ comprising Rho kinase inhibitor has been submitted (EPO 956 865 A1). This inhibitor has not been tested for efficacy in CNS injury, nor has the company who patented this compound discovered how it might [[to]] be applied to a region of CNS injury in a kit form. Such a kit is provided in our invention. Please see also European Patent application no. 97934756.4; PCT/JP97/02793; International publication # WO 98/06433 (19.02.1998/07).

- Please replace the paragraph found at page 12 from lines 1 to 11 with the following paragraph:

As discussed herein in accordance with the present invention a therapeutically active agent for facilitating axon growth may be delivered (in a flowable matrix forming substance) to a (nerve) lesion site, for example, by injection using known syringe type glue or sealant devices modified as necessary or desired (e.g. by addition of a further substance container); examples of known delivery devices, systems, mechanisms, matrix forming compositions, and ~~teh~~ the like are shown for example in U.S. patent no. 5,989215, U.S. patent no. 4,978,336, U.S. patent no. 4,631,055, U.S. Pat. No. 4,359,049, ~~U.S. Pat. No. 4,974,368~~, U.S. patent no. 6,121,422, U.S. patent no. 6,047,861, U.S. patent no. 6,036,955, U.S. patent no~~. 5,945,1115~~ 5,945,115, U.S. patent no. 5,900,408, U.S. patent no. 6,124,273, U.S. patent no. 5,922,356, and in particular U.S. patent no. 6,117,425; the entire contents of each of these patents is incorporated herein by reference.

- Please replace the paragraph found at page 18 from lines 28 to 30 with the following paragraph:

Figure 1A is a schematic diagram of adhesive delivery system of C3 applied to an injured spinal cord wherein a tissue adhesive plus Rho antagonist (i.e. C3) is ~~njected~~ injected into the site of ~~ninjury~~ injury;

- Please replace the paragraphs found at page 19 from lines 1 to 18 with the following paragraphs:

Spinal cord wherein the injection is shown as resulting in axon regeneration ~~thrugh~~ through the supplemented adhesion matrix and into the distal spinal cord;

Figure 2 Schematically illustrates the model used to show efficacy in vivo. A dorsal hemisection was made in adult mice. Three to four weeks later the anterograde tracer WGA-HRP was injected into the cortex to label the neurons of the corticospinal tract. Two days later the spinal cord was removed and [[and]] HRP enzymatic activity revealed to detect the CST axons. The corticospinal tract of adult mice was ~~lesion~~ lesioned at the T6 level, and the fibrin glue/C3 was added at the time of lesion with a syringe. The ~~expresssion~~ expression CST ~~referes~~ refers to cortical spinal tract.

Figure 3 Illustrates a longitudinal section of an untreated adult mouse spinal cord 3 weeks after lesion of the corticospinal tract viewed by darkfield microscopy. The fibers were anterogradely labeled from the motor cortex and appear fluorescent. The ~~fibres~~ fibers retract back from the site of lesion and do not regenerate with treatment.

- Please replace the paragraphs found at page 19 from lines 25 to 32 with the following paragraphs:

Figure 4C Illustrates a low magnification view of ~~labelled~~ labeled corticospinal

axons near the lesion site after treatment with collagen gel with C3 as a Rho antagonist; axons do not retract ~~bak~~ back from the lesion site; ~~thy~~ they extend into the region of increased cellularity which is the scar;

Figure 4D Illustrates a higher magnification view of Figure C showing that treatment with Rho ~~antagonst is~~ antagonist in a collagen gel allows some axons to sprout into the lesion site;

- Please replace the paragraphs found at page 20 from lines 1 to 15 with the following paragraphs:

Figure 5A Illustrates a low magnification view of a spinal cord following treatment with fibrin adhesive with C3 as a Rho antagonist; the section is viewed by darkfield to show the ~~anterogradley~~ anterogradely-labeled ~~fibres~~ fibers that appear white;

Figure 5B Illustrates a ~~hgh~~ high magnification view of the lesion site shown in Figure 5A showing that axons grow through the scar region; the scar appears as the ~~verticle~~ vertical line;

Figure 5C Illustrates a ~~hgh~~ high magnification view approximately 7mm distal ~~o the lsion ste~~ of the lesion site of the spinal cord shown in Figure 5A and 5B; the regenerating ~~fibres~~ fibers (arrows) grow long distances;

Figure 6A Illustrates a darkfield microscopy of a spinal cord section after treatment with Rho antagonist C3 in a fibrin adhesive ~~showning~~ showing long distance regeneration; axons sprout into the white matter and cross the lesion site;

- Please replace the paragraph found at page 20 from lines 20 to 22 with the following paragraph:

Figure 7A Illustrates an untreated ~~mose~~ mouse two days after spinal cord injury; the control mouse is mobile but uses its front paws to drag itself forward ant it shows some movement of hindlimb joints;

- Please replace the paragraph found at page 20 from lines 28 to 32 with the following paragraph:

Figure 7C Illustrates a comparison of fibrin, collagen, ~~Gelfoam~~ Gelfoam™ and ~~Elvax~~ Elvax™ methods of C3 delivery on long-distance regeneration. Animals were treated with the test delivery system without (-C3) or with (+C3) Rho antagonist. Distance of growth of the longest axon was scored by blind examination of at least five sections from each animal. The longest distance of axon growth was scored.

- Please replace the paragraphs found at page 32 from lines 1 to 32 with the following paragraphs:

before application, so that polymerization of the gel occurs in the injured CNS. Therefore, it is important that the fibrinogen and thrombin are ~~package~~ packaged separately. However, the C3 can be packaged separately, or added to either the thrombin or fibrinogen bottles. In another formulation, the fibrinogen, thrombin and C3 are packaged together, but ~~help~~ held at low pH, which prevents polymerization of the gel. Polymerization would be induced by mixing this ~~formution~~ formulation with a basic component that would neutralize the pH to induce coagulation of the adhesive. In another ~~formultion~~ formulation, the Rho ~~antogonist~~ antagonist could be added separately to the fibrinogen/thrombin mix in the form of liposomes or other similar delivery system. Living cells ~~could that~~ that could secrete C3 could be added as Rho antagonist.

A method of Applying Rho antagonist in vivo is discussed hereinafter.

Tissue adhesive formulations are typically applied to wound sites with a syringe and needle. The shape of the ~~need determine~~ needle determines the type of surface that is formed when the adhesive polymerizes. In some cases, adhesives can be sprayed onto the wound surface, or into the desired region. This invention covers all types of syringes and needles used to apply fibrin plus Rho antagonists to injured regions of the CNS. In addition, it covers the addition of previously polymerized tissue adhesives with C3 to the wound. ~~Forexample~~ For example, fibrin can be polymerized in a ~~teat~~ test tube, and ~~forcepts~~ forceps used to remove the gel and place it in the body cavity. Similarly, collagen can be applied by pre-polymerization and application by using ~~focepts~~ forceps to place the gel in the injured spinal cord. One example of this is more fully explained in the example section of this application.

Tests were done with Gelfoam(TM), a surgical collagen-based sponge, and ~~Elvax~~ Elvax™, a slow release plastic (Lehmann et al 1999, IBID) for the ability to deliver biologically effective concentrations of C3. Neither of these two delivery systems was effective. Therefore, only tissue adhesive formulations (i.e. the matrix forming formulations discussed herein) have efficacy in the delivery of C3 to the injured CNS in vivo.

Therapeutic Applications/Medical Uses will be discussed below.

The tissue adhesive system for the delivery of Rho ~~antoagonists~~ antagonists may be useful in many other

- Please replace the paragraphs found at page 33 from lines 1 to 12 with the following paragraphs:

Conditions that effect the central and paripheral nervous system. Treatments that are effective in eliciting sprouting from injured axons are equally effective in trating some types of stroke (Boston life sciences, Sept. 6, 200 Press Release). Since it has been

determined that it is possible to elicit sprouting (using ~~akit~~ a kit of the present invention), it is obvious that the treatments can be extended to stroke. Similarly, altough the subject of this invention is related to delivery of Rho antagonist to the traumatically damaged nervous system, this invention also pertains to damage from neurodegeneration, such as during Parkinson's disease, Alzheimer's disease, prion diseases or other diseases of the CNS ~~were~~ where axons are damaged, in the CNS environment. In such cases, small volumes of the tissues adhesive with C3 could be injected into the affected region with the use of a syringe. The treatment will cause local sprouting to restore function of neurons whose axon processes had retracted in the course of the neurodegeneration.

- Please replace the paragraph found at page 33 from lines 28 to 32 with the following paragraph:

The kit contains:
1 vial ~~fibrigen~~ fibrinogen
1 vial ~~apropinin~~ aprotinin solution for ~~reconsitution~~ reconstitution of fibrinogen
1 vial thrombin
1 vial calcium chloride solution for ~~reconsitution~~ reconstitution of thrombin

- Please insert the following passage after page 11;

The present invention provides in one aspect thereof, an axon growth stimulation kit which may comprise a first container means for containing a flowable carrier component or two or more separate components capable once intermingled of forming a flowable carrier component, the flowable carrier components each being capable of forming a therapeutically acceptable matrix in vivo at a nerve lesion site and a second container means for containing a therapeutically active agent for facilitating axon growth at the lesion site, wherein the therapeutically active agent may be releasable from the in vivo matrix into the adjacent external environment.

More particularly, the present invention provides an axon sprouting (growth) stimulation kit which may comprise

a first container means which may have a first matrix forming element, and;

a second container means which may have a second matrix forming elements, the first and second matrix forming elements being capable once intermingled of forming a flowable carrier component and the first and second matrix forming elements further being capable of forming a therapeutically acceptable in vivo fibrin matrix at a nerve lesion site,

and at least one of the first and second container means may further comprise a therapeutically active agent selected from the group consisting of C3 and Y-27632 for facilitating axon sprouting (growth) at the lesion site and wherein the therapeutically active agent may be releasable from the therapeutically acceptable in vivo fibrin matrix into an adjacent external environment.

Also, more particularly the present invention provides an axon sprouting stimulation kit which may comprise

- a first container means which may have a first matrix forming element, and;
- a second container means which may have a second matrix forming element, the first and second matrix forming elements being capable once intermingled of forming a flowable carrier component and the first and second matrix forming elements further being capable of forming a therapeutically acceptable in vivo fibrin matrix at a nerve lesion site, and;

-a third container means which may comprise a therapeutically active agent selected from the group consisting of C3 and Y-27632 for facilitating axon (growth) sprouting at the lesion site,

wherein the therapeutically active agent may be releasable from the therapeutically acceptable in vivo fibrin matrix into an adjacent external environment.

The axon sprouting (growth) stimulation kit may comprise means for dispersing the therapeutically active agent in the flowable carrier component so as to form a flowable axon sprouting (growth) stimulation composition

and means for delivering the flowable axon sprouting (growth) stimulation composition to the lesion site.

In a further aspect, the present invention provides a biocompatible composition which may comprise: (i) at least one supplement selected from the group consisting of therapeutically active agents for facilitating axon growth; and (ii) a flowable carrier component being capable of forming a therapeutically acceptable matrix in vivo at a nerve lesion site, wherein the supplement may be releasable from the matrix into the adjacent external environment.

More particularly, in accordance with the present invention, there is provided a biocompatible composition which may comprise: (i) a therapeutically active agent selected from the group consisting of C3 and Y-27632 for facilitating axon sprouting (growth), and (ii) a first matrix forming element being capable of forming a flowable carrier component once intermingled with a second matrix forming element, and the first and second matrix forming elements further being capable of forming a therapeutically acceptable in vivo fibrin matrix at a nerve lesion site, wherein the therapeutically active agent may be releasable from the in vivo fibrin matrix into an adjacent external environment.

In yet a further aspect, the present invention provides a method for the preparation of a flowable biocompatible composition which may comprise admixing (i) at least one supplement selected from the group consisting of therapeutically active agents for facilitating axon growth and (ii) a flowable carrier component being capable of forming a therapeutically acceptable matrix in vivo at a nerve lesion site; wherein the supplement may be releasable from the matrix into the adjacent external environment.

More particularly, in accordance with the present invention, there is provided a method for the preparation of a flowable biocompatible composition which may comprise admixing (i) a therapeutically active agent selected from the group consisting of C3 and Y-27632 for facilitating axon sprouting (growth), and (ii) a first and second

matrix forming elements being capable once intermingled of forming a flowable carrier component and the first and second matrix forming elements being capable of forming a therapeutically acceptable in vivo fibrin matrix at a nerve lesion site, wherein the therapeutically active agent may be releasable from the in vivo fibrin matrix into the adjacent external environment.

The present invention also relates to a flowable biocompatible composition obtained from the method described herein.

In accordance with the present invention, the therapeutically acceptable matrix may be a collagen matrix or a fibrin matrix. Also, in accordance with the present invention, C3 may be selected, for example, from the group consisting of an ADP-ribosyl transferase C3 derived from Clostridium botulinum, a C3 analogue capable of inactivating a Rho GTPase and a recombinant ADP-ribosyl transferase C3.

- Please insert the following passage at page 21, line 17;

Figure 10 Is a schematic diagram of another embodiment of a system exploiting a kit in accordance with the present invention.

- Please insert the following passage at page 46, line 3;

Turning now to Figure 10, this figure illustrates in schematic fashion another embodiment of a system exploiting a kit of the present invention for mixing and delivering a supplemented matrix forming material. The containers means are as described in Figure 9 with the exception that the "container means for therapeutically active agent" (container means 3) has been interchanged with the "container means for second matrix forming element" (container means 1). Feed line 10 is indicative of the addition of a first matrix forming element from container 2 with C3 from container 3 into a mixing container 6. Feed line 8 is indicative of the addition of a second matrix forming element into container 6. Mixing may be effected as described for figure 9.